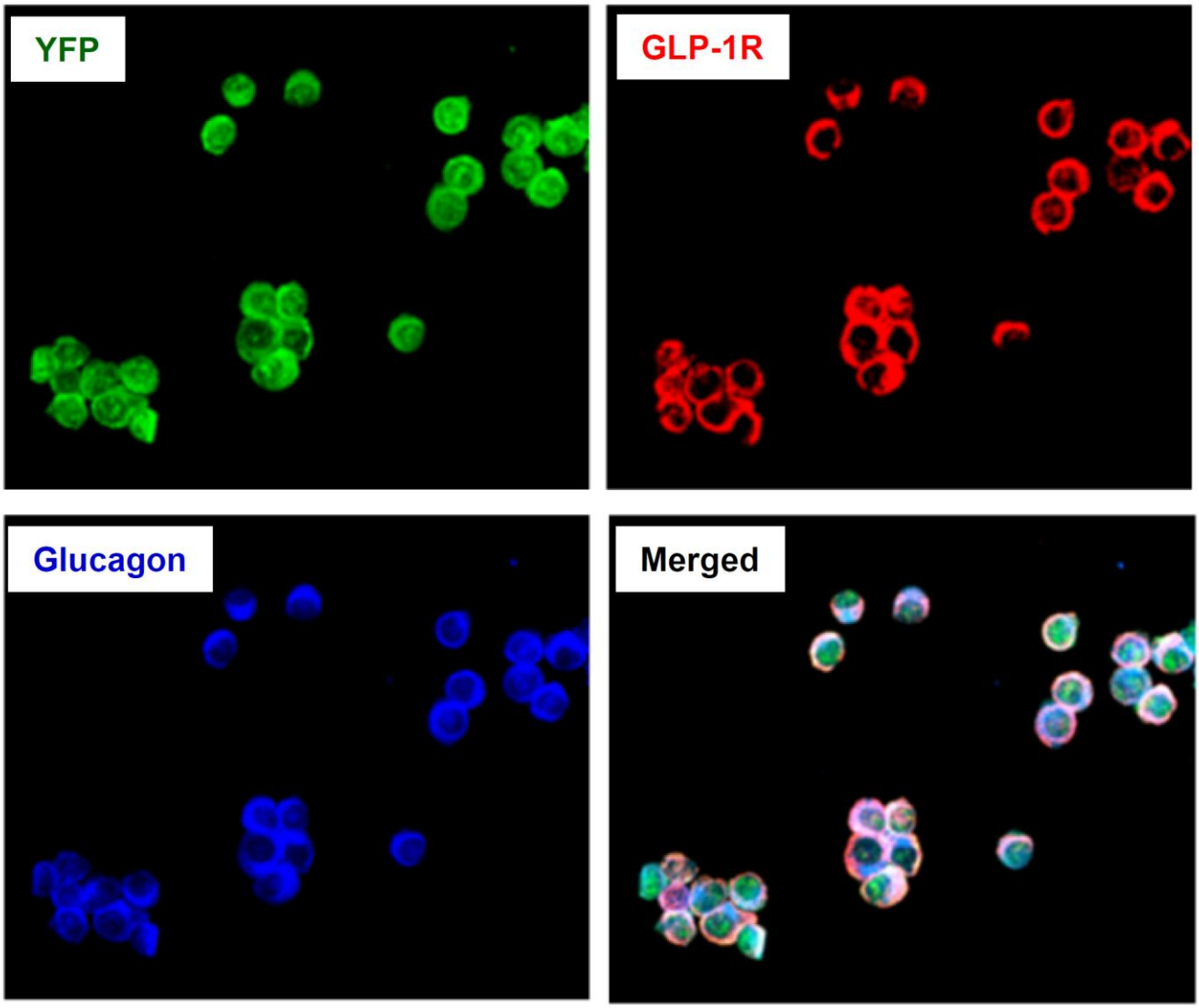
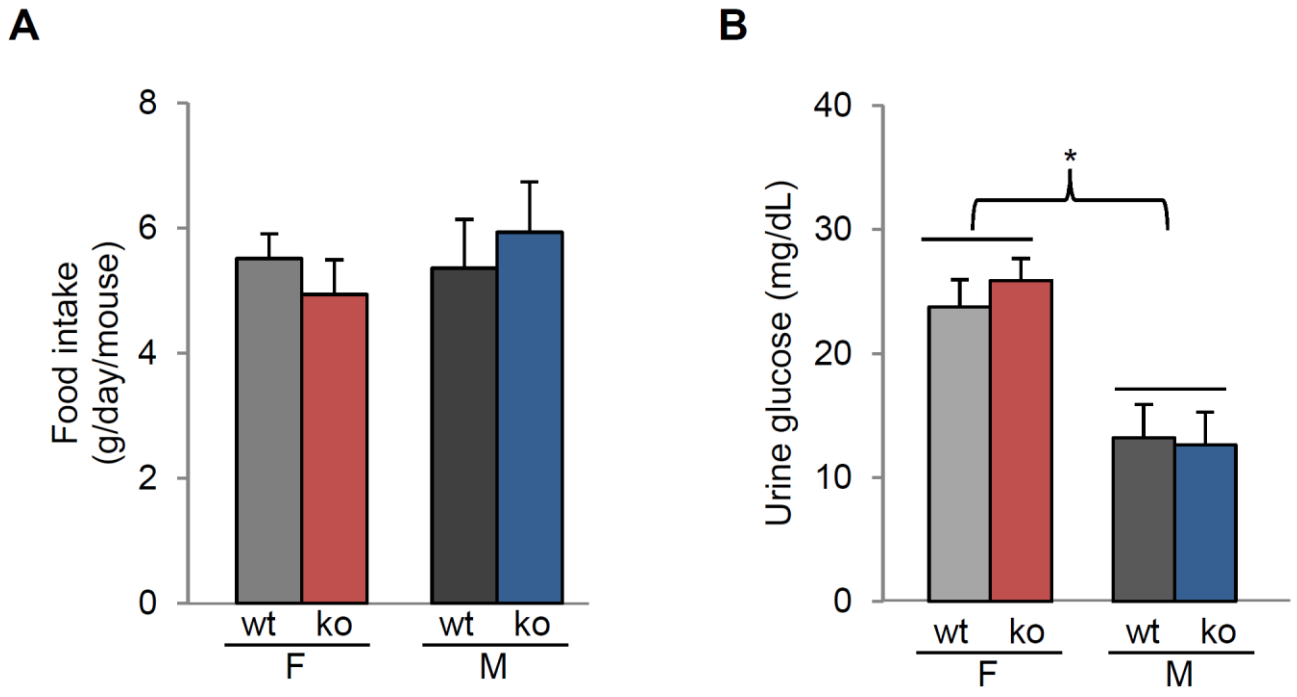
SUPPLEMENTARY DATA

Supplementary Figure S1. GLP-1R expression in FACS-purified α cells from αYFP mice. FACS-purified α cells were co-stained for YFP (green), GLP-1R (red) and glucagon (blue) with corresponding antibodies, and then imaged by confocal microscopy. The data showed that all cells are YFP^+ α cells and all expressed GLP-1R.



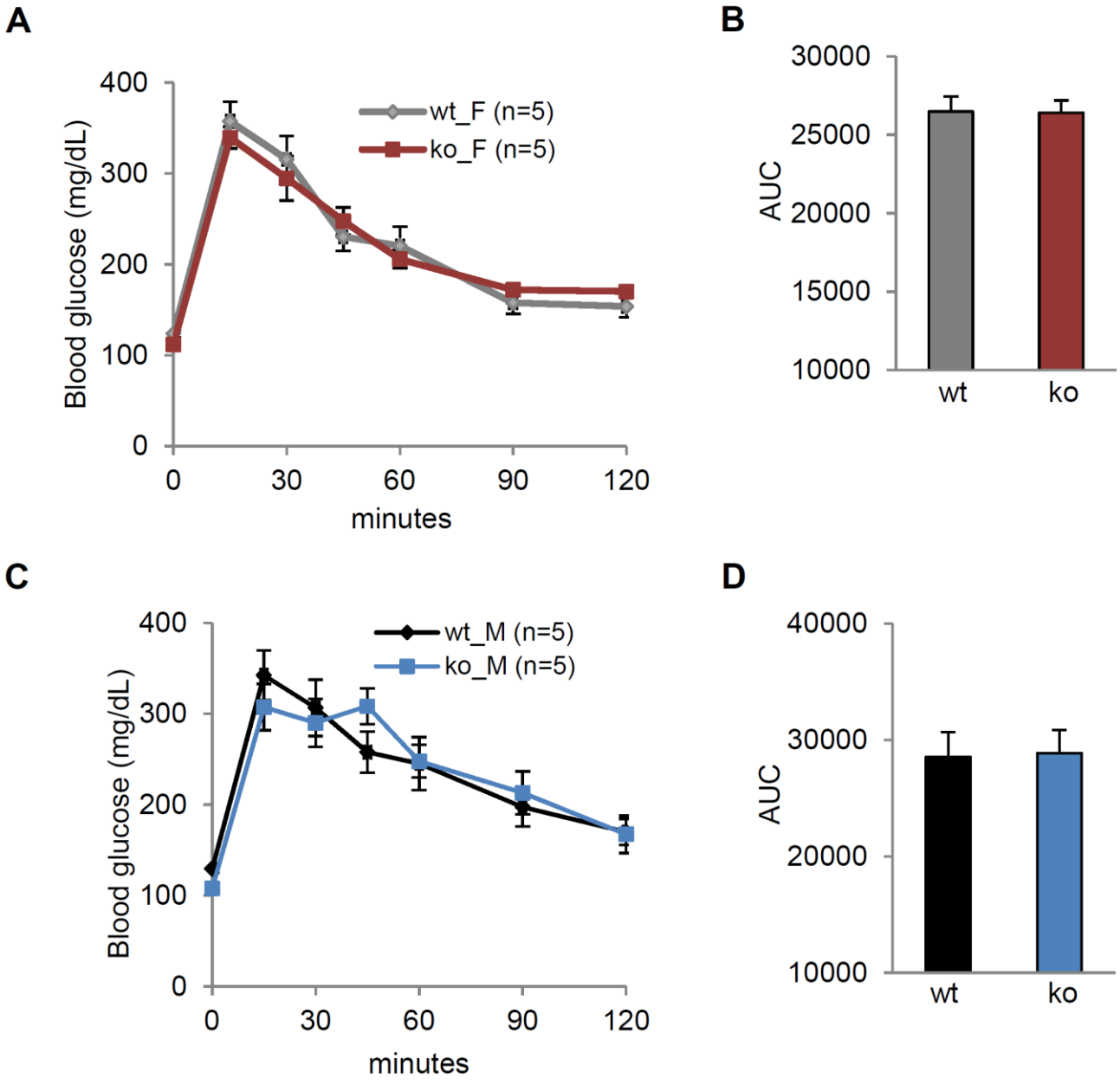
SUPPLEMENTARY DATA

Supplementary Figure S2. Food intake and urine glucose levels of the αGLP-1R$^{-/-}$ (ko) and control (wt) mice. (A) Food intake of the mice (3-4 months old, n=8-10) was recorded for 5 continuous days. No significant difference was detected between the wt and ko mice, for either females (F) or males (M). (B) Urine glucose levels of the ko and wt mice. Urine (random, non-fasting) was collected from the ko and wt mice ((3-4 months old, n=8-10). Glucose concentrations in the urine were measured with Mouse Glucose Assay kit (Crystal Chem, Elk Grove Village, IL). The ko mice and their wt littermates showed similar glucose levels in the urine. However, the females had significantly higher urine glucose levels than the males, for both wt and ko mice, suggesting there is a gender difference on urine glucose disposal which does not appear to be related to αGLP-1R knockout. *: $p<0.05$.



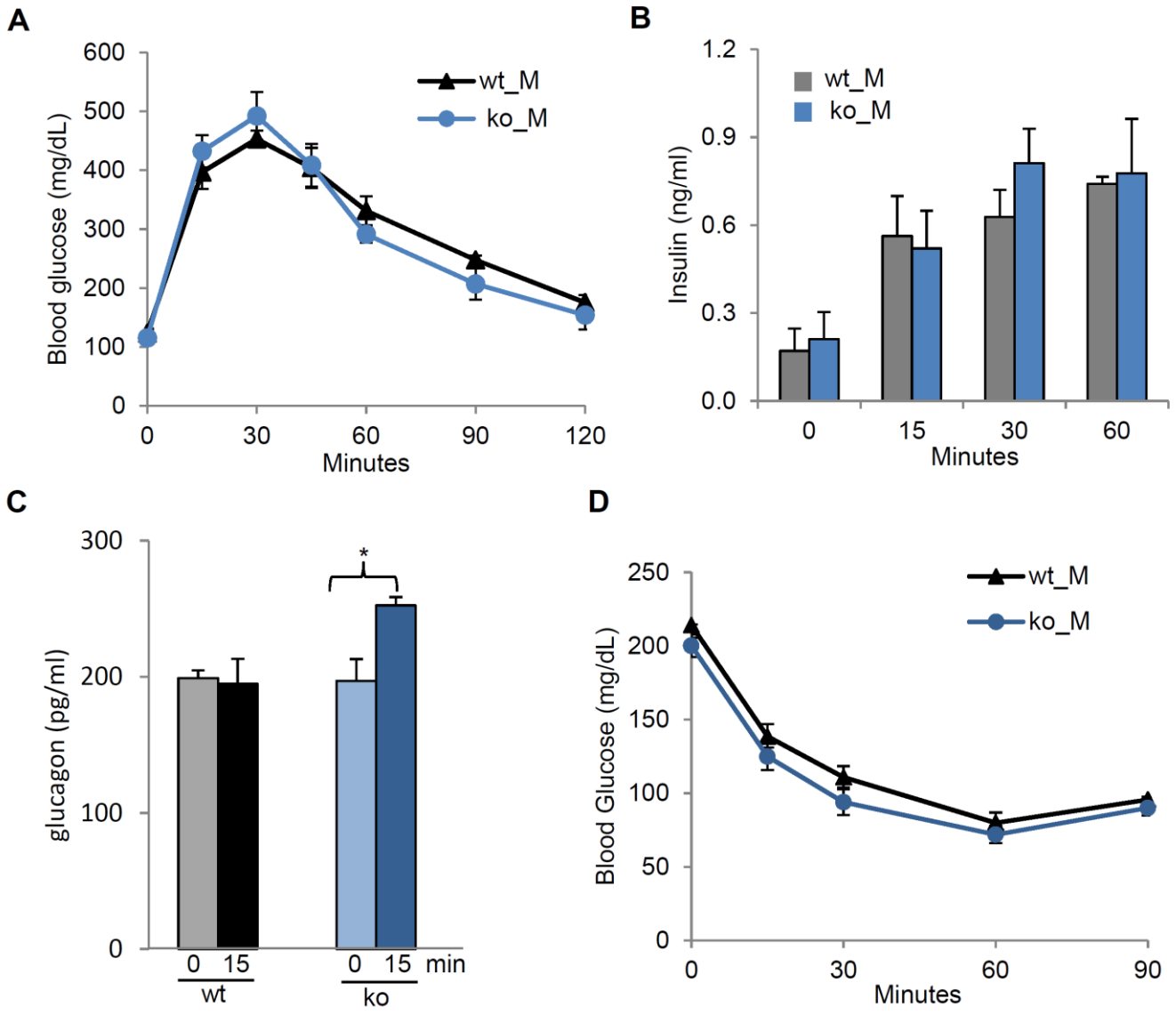
SUPPLEMENTARY DATA

Supplementary Figure S3. Oral glucose tolerance test (OGTT) showed no significant differences between the αGLP-1R$^{-/-}$ mice and the control littermates. Following overnight fasting, the mice (~4 months old, n=5 per group) were orally administered with 2g glucose/kg bodyweight, and their blood glucose were monitored for the next 2 hours. Both female (A-B) and male (C-D) mice were tested. The ko and wt mice showed essentially the same response to the OGTT.



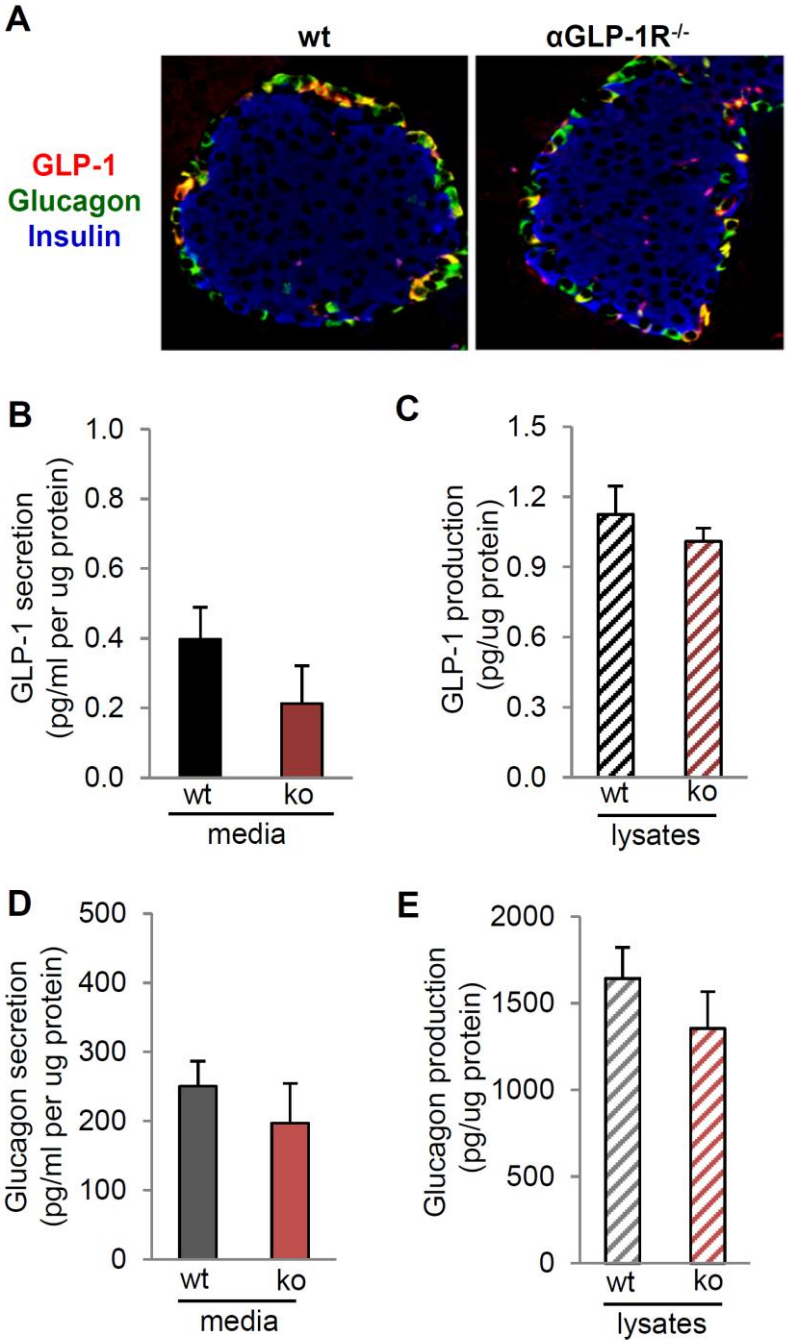
SUPPLEMENTARY DATA

Supplementary Figure S4. Physiological effect of αGLP-1R knockout on male mice. Male αGLP-1R$^{-/-}$ (ko) mice and their wildtype (wt) littermates, 4-6 months old, were used in these assays. (A) i.p. Glucose Tolerance Test (ipGTT) following overnight fasting, with 2g/Kg body weight of glucose, n=8-10 (mice/group). (B) insulin secretion in the same setting as ipGTT, n=8-10. (C) Glucagon secretion in the same setting as ipGTT, n=8-10. Blood was collected prior to glucose injection (time 0), and 15 minutes after glucose injection. *: $p<0.05$. (D) Insulin Tolerance Test (ITT) with 0.75U insulin/Kg bodyweight following 6 hours fasting, n=5. Of note: Glucose stimulated glucagon secretion in the male αGLP-1R ko mice, similar to what observed in the female ko mice. However, this did not result in glucose intolerance in male mice, suggesting the males are more resistant to glucagon dysregulation than females.



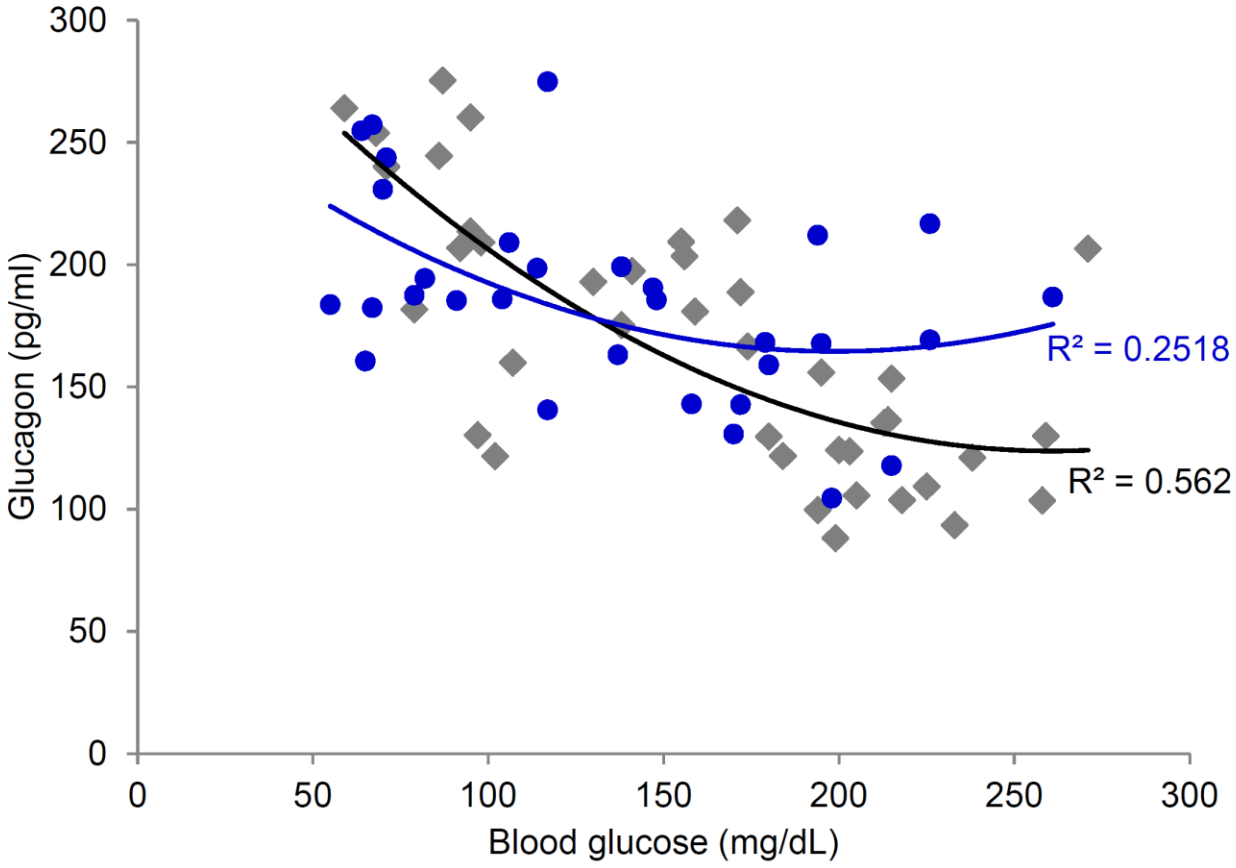
SUPPLEMENTARY DATA

Supplementary Figure S5. αGLP-1R deletion did not affect intra-islet GLP-1 production and secretion. (A) Immunohistochemistry assessment of intra-islet GLP-1 expression. Pancreatic slices from wild-type (wt) and αGLP-1R$^{-/-}$ (ko) mice (4 months old) were co-stained with insulin, glucagon, and GLP-1 antibodies. The anti-GLP-1 antibody (Abcam) was specific for the active form of GLP-1 (amidated GLP-1$_{7-36}$). Detection of glucagon only (green) and GLP-1 only (red) cells confirmed the specificity of the antibodies. (B-E) Evaluation of GLP-1 vs glucagon production and basal secretion in isolated islets. Freshly isolated islets from the αGLP-1R$^{-/-}$ and wt mice (n=6) were cultured for two days in complete islet culture media. The media and islet lysates were collected and processed for hormone measurements. Basal GLP-1 secretion (B) and glucagon secretion (D) were measured using the culture media; and intra-islet GLP-1 production (C) and glucagon production (E) were measured using the islet lysates. All data were normalized with total islet protein (per ug protein). No significant differences were detected between the groups for each of the parameters.



SUPPLEMENTARY DATA

Supplementary Figure S6. The relationship between glucagon secretion and blood glucose in male αGLP-1R$^{-/-}$ (ko) and wt mice. Circulating glucagon concentrations of male mice were plotted against their blood glucose levels. Each gray diamond represents a wt mouse, and each blue circle represents a ko mouse. The various blood glucose levels were achieved through extended fasting (~40 hrs), overnight fasting (~16 hrs), non-fasting (random), or feeding/postprandial (1 hr after lights-off) conditions. The best-fit Polynomial (order 2) trendlines and their corresponding R^2 values were displayed. Gray: wt; Blue: ko.



SUPPLEMENTARY DATA

Supplementary Figure S7. Analysis of free amino acids in the blood of the αGLP-1R$^{-/-}$ mice and the control littermates. Non-fasting blood samples from wt and αGLP-1R$^{-/-}$ mice (n=3, females) were processed for free amino acid analysis by Biosynthesis, Inc (Lewisville, TX). (A) A table showing the concentrations of each amino acid in the wt and ko mice. Also included are some common amino acid derivatives. The data are expressed as Mean ± SEM. Most of the amino acids in the ko mice showed a trend of increase compared to the wt mice, and several of them showed statistically significant differences. *: $p<0.05$; n.d.: not detectable. (B) The total amount of amino acids in the blood of αGLP-1R$^{-/-}$ mice was significantly higher than that of the wt mice.

A

Amino Acid (AA)	wt (ug/ml)	αGLP-1R KO (ug/ml)	*p* value
D	4.73 ± 1.11	4.65 ± 1.80	0.970
T	**30.23 ± 1.26**	**42.15 ± 4.05**	**0.048** *
S	26.02 ± 3.05	35.77 ± 1.95	0.054
N	8.82 ± 1.12	10.92 ± 0.26	0.143
E	13.85 ± 1.98	14.62 ± 4.47	0.883
Q	180.77 ± 16.95	217.00 ± 17.88	0.215
P	14.52 ± 0.97	22.57 ± 4.20	0.135
G	**27.50 ± 2.72**	**40.37 ± 1.82**	**0.017** *
A	**59.55 ± 3.84**	**91.87 ± 8.78**	**0.028** *
V	41.30 ± 4.16	46.25 ± 3.80	0.429
M	16.73 ± 2.30	22.83 ± 4.32	0.281
C	n.d.	n.d.	n.d.
I	17.33 ± 0.92	20.97 ± 2.17	0.198
L	27.83 ± 2.05	34.38 ± 3.86	0.208
Y	18.52 ± 2.73	23.88 ± 1.08	0.141
F	20.83 ± 0.81	25.55 ± 1.51	0.052
W	39.57 ± 3.87	41.82 ± 1.45	0.615
K	73.42 ± 3.51	87.87 ± 7.23	0.147
H	15.37 ± 2.25	22.38 ± 2.69	0.116
R	54.48 ± 2.22	66.62 ± 8.64	0.245
ethanolamine	2.92 ± 0.22	2.50 ± 0.61	0.553
α-Amino Butyric Acid	2.08 ± 0.35	5.32 ± 3.65	0.428
citrate	**20.33 ± 1.03**	**26.17 ± 1.79**	**0.048** *
creatine	2.50 ± 0.58	4.25 ± 0.55	0.153
taurine	163.57 ± 5.77	172.62 ± 25.98	0.751

B